

FiberCell Systems Inc.
a better way to grow cells

CDM-HD Usage Instructions

CDM-HD (chemically defined medium for high density cell culture) is a chemically defined, protein free, animal component free cell culture supplement that is designed to replace fetal bovine serum in standard basal cell culture mediums such as DMEM. CDM-HD is designed specifically for high-density cell culture and should be used in the FiberCell® Systems hollow fiber system for best results. Cells cultured in a hollow fiber bioreactor are typically 100X the density of cells grown in flasks or other conventional cell culture devices. Cells grown at these densities are able to auto-support their growth with secreted conditioning factors and can be grown efficiently with a simplified serum replacement like CDM-HD. It is important to know both what is IN CDM-HD and what is NOT.

CDM-HD contains:

- 1) A complex formulation of vitamins, micronutrients, amino acids and other basic chemicals
- 2) A significant amount of free iron
- 3) The equivalent of 10mM HEPES per liter when added at 10% to medium
- 4) The equivalent of 1 gram of glucose per liter when added at 10% to medium



CDM-HD does NOT contain:

- 1) Protein of any sort. Therefore there are neither attachment factors nor iron transport proteins.
- 2) Surfactants like pluronic F-68
- 3) Animal or plant hydrolysates or anything else of an undefined nature.

CDM-HD may also be used in spinner or shaker flask culture, if desired. There are no surfactants added to CDM-HD because in hollow fiber there is little or no shear on the cells. Spinner and shaker flask do contribute to shear on the cells and it is recommended that a surfactant such as pluronic F-68 be added to the medium to protect the cell membranes. Because the cells are at a lower density you may need to use a higher concentration of CDM-HD such as 15-20%. Since there are no attachment factors you will need to seed some adherent cells in the presence of serum, allow them to attach and then switch to CDM-HD.

For use in the FiberCell® Systems hollow fiber bioreactor system it is recommended that the cells be inoculated into the cartridge in the presence of at least 5% serum in the cell inoculum. Once the cells

have reached high density in the cartridge, as defined by a glucose uptake rate of 1 gram per day, simply switch out the medium in the reservoir and ECS for medium containing 10% CDM-HD. You may observe a slight lag in growth of the cells for a day or two but no sequential adaptation is required.

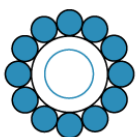
Protein purification using CDM-HD: Since CDM-HD is protein free you may be able to simplify your purification protocols. The higher than normal amount of iron can cause precipitation when the supernatant harvests are placed directly into a phosphate buffer. When using a phosphate buffer it is recommended that you add 10mM EDTA to the bulk harvest or reduce the iron content by dialyzing against a citrate or EDTA containing buffer before continuing.

Preparation of CDM-HD for use.

1. Fill a mixing vessel with distilled or WFI quality water, to 90% of the desired total volume of media. For example, for one liter of CDM-HD, fill the vessel to 900mL.
2. Add CDM-HD to the water while gently stirring. Rinse the inside of the package with some of the remaining 10% water to remove any remaining powder.
3. Finish adding remaining water to bring to a volume of 1 liter and mix until completely dissolved. This should take 15-30 minutes at room temperature.
4. CDM-HD should be pH adjusted to 6.8 if necessary, using 1NaOH or 1N HCL.
5. Filter into a sterile container by membrane filtration, using a 0.2 micron filter.
6. Store at 4-6 C.
7. CDM-HD is provided at 36 grams to make one liter.

Notes:

- 1) Do not heat CDM-HD to speed dissolution.
- 2) pH should be adjusted to no higher than 6.8 for best storage. CDM-HD is stable as a liquid at 4 degrees for at least 6 weeks.
- 3) Do not freeze CDM-HD once it has been reconstituted.
- 4) CDM-HD powder should be stored at 4 degrees C and has a shelf life of at least one year.
- 5) Add CDM-HD to classical media such as DMEM High glucose at a final concentration of 10%.



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